

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Ichiro HIRAO *et al.*

Application No.: 10/521,454

Confirmation No.: 8799

Filed: November 29, 2005

Art Unit: 1633

For: NUCLEOSIDE OR NUCLEOTIDES HAVING
NOVEL UNNATURAL BASES AND USE
THEREOF

Examiner: J.L. Epps Ford

37 C.F.R. § 1.132 DECLARATION OF ICHIRO HIRAO

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Commissioner for Patents
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Sir:

I, Ichiro Hirao, declare the following:

I am a co-inventor of the above-identified application.

My education and career are as follows:

1978 B.Eng.	Department of Industrial Chemistry, Faculty of Engineering, Shizuoka University
1983 Ph.D.	Department of Chemistry, Faculty of Science, Tokyo Institute of Technology
1984-1992	Assistant professor, Department of Industrial Chemistry, Faculty of Engineering, The University of Tokyo
1992-1996	Associate professor, Laboratory of Pharmaceutical Chemistry, Tokyo College of Pharmacy

1995-1997	Associate Scientist, Department of Chemistry, Indiana University
1997-2001	Group Leader, Yokoyama CytoLogic Project, ERATO, Japan Science and Technology Corporation
2001-2002	Team Leader, Protein Preparation/NMR Facilities, RIKEN Genomic Sciences Center
2002-2006	Professor, Research Center for Advanced Science and Technology, The University of Tokyo
2002-2006	Senior Visiting Scientist, Protein Research Group, RIKEN Genomic Science Center
2006-2008	Team Leader, Protein Preparation/NMR Facilities, RIKEN Genomic Sciences Center
2007-present	CEO, TagCyx Biotechnologies Co.
2007-present	Visiting Professor, Graduate School of Engineering, Hokkaido University
2008-present	Team Leader, Nucleic Acid Synthetic Biology Research Team, Systems and Structural Biology Center (SSBC), RIKEN

I have reviewed the above-identified application, and in particular the Office Action dated July 11, 2008 and the references cited therein.

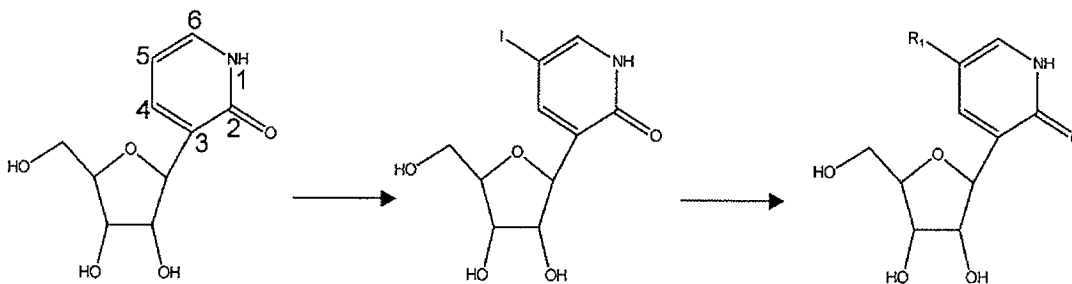
Novelty and Unexpected Results of the Present Invention

The present invention provides a nucleoside or nucleotide having a 5-substituted -2-oxo(1H)-pyridin-3-yl group as a base, wherein the 5-position of the base is substituted with a particular substituent selected from the group consisting of 1) to 4) described in Claim 2 of the present application. It is submitted that the method of synthesizing the nucleoside or nucleotide of the present invention is novel. Ohtsuki *et al.*, "Unnatural Base Pairs for Specific Transcription," *Proc. Natl. Acad. Sci.*, Vol. 98, (2001), pages 4922-4925 (hereinafter "Ohtsuki

et al.”), and Froehler *et al.*, U.S. Patent No. 6,447,998, U.S. Patent No. 6,495,672 or US Patent Publication No. 2003/0120065 (hereinafter “Froehler *et al.*”), or combinations thereof, would not provide a method that would enable those skilled in the art to synthesize 5-substituted-2-pyridone derivatives of the present invention. The nucleoside or nucleotide of the present invention is, therefore, novel, and is not easily obtained even by referring to the prior art documents. It is submitted that the nucleoside or nucleotide of the present invention first enabled those skilled in the art to introduce nucleosides having the 2-pyridone derivatives with various substituents at the 5-position into a specific position in DNA or RNA by replication or transcription mediated by artificial, extra base pair systems.

Novelty of the nucleoside or nucleotide of the present invention

(1) The present invention

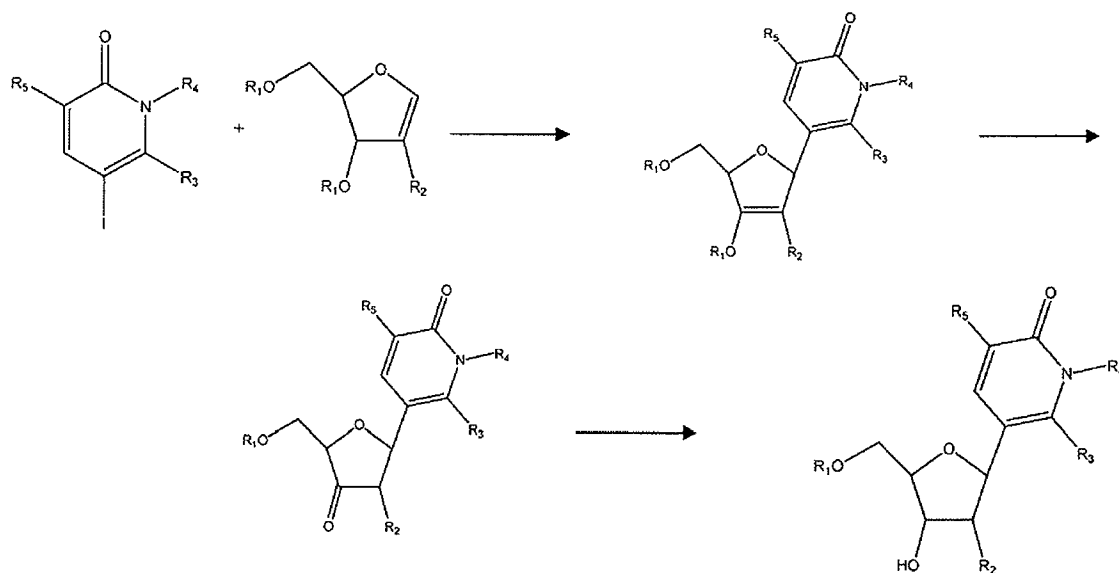


The method for synthesizing the nucleoside derivatives of 5-substituted-2-pyridone of the present invention comprises first synthesizing the nucleoside of 2-pyridone, iodinating the 5-position of the 2-pyridone moiety, and then introducing various substituents, preferably through linkers, such as alkyne, into the 5-position. Ohtsuki *et al.* and Froehler *et al.* do not disclose or suggest any method to enable synthesis of the various 5-substituted derivatives. The present

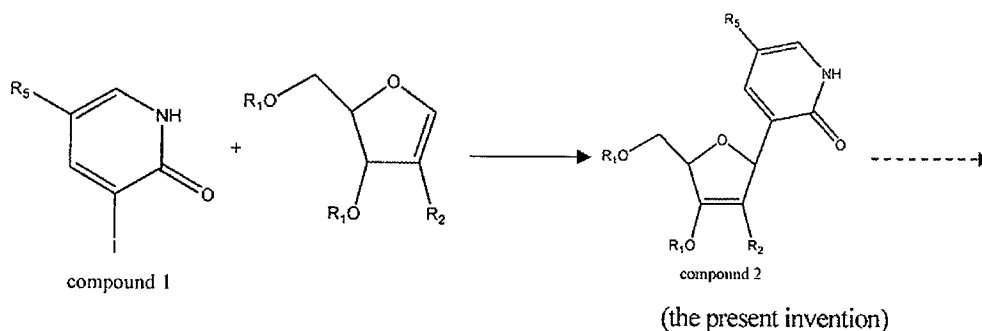
specification provides for the first time a possible method to synthesize a wide variety of 2-pyridone derivatives with various useful substituents at the 5-position.

One of the technical points of the present method is to selectively iodinate the 5-position of 2-pyridone. One could expect that all of the 4-, 5- or 6-positions of the 2-pyridone moiety might be iodinated. However, it has been demonstrated for the first time that only the 5-position is selectively reacted and iodinated. Accordingly, the nucleoside derivatives of the 5-substituted-2-pyridone of the present invention were obtained only after developing the synthesizing method disclosed in the present specification.

(2) The invention of Froehler *et al.*



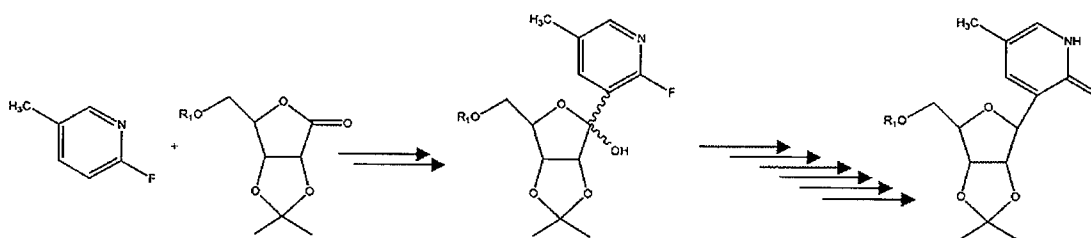
Froehler *et al.* describe a method to synthesize nucleoside derivatives having pyridone with substituents, such as an alkyl group and alkynyl group at R5, wherein the method includes 3 steps comprising reactions which bind 5-iodo-2-pyridone to a ribose. In the method of Froehler *et al.*, the substituent at R3 is defined as to -H or -CH₃. Therefore, the method is completely different from the method for synthesizing the nucleoside derivatives of the present invention having 2-pyridone (the position corresponding to R3 is keto group).



Compound 2 of the above reaction having a halogen at the 5-position (R₅) is one of the present nucleoside derivatives of the present invention. It is also an important intermediate for further synthesizing various 5-substituted 2-pyridones. However, it is noteworthy that Compound 2 having a halogen at 5-position cannot be synthesized by the method disclosed in Froehler *et al.* This is because the yield of a radical at the 2-iodo position of the starting material, 2-halopyridone, is necessary to react with a ribose material via addition reaction. Thus, the halide compound 1 (R₅ = halogen) cannot be used for the glycosidation reaction since the R₅ position, as well as the 2-iodo position, would also react with the ribose material. It is also evident that

Froehler *et al.* describe only simple substituents, such as an alkyl group or alkynyl group, but does not describe or suggest more complicated substituents, including compounds where R5 is halogen. There was no report before the present invention that the present nucleoside derivatives having various substituents at the 5-position of 2-pyridone have actually been synthesized by the method of Froehler *et al.*

(3) The invention of Ohtsuki *et al.*



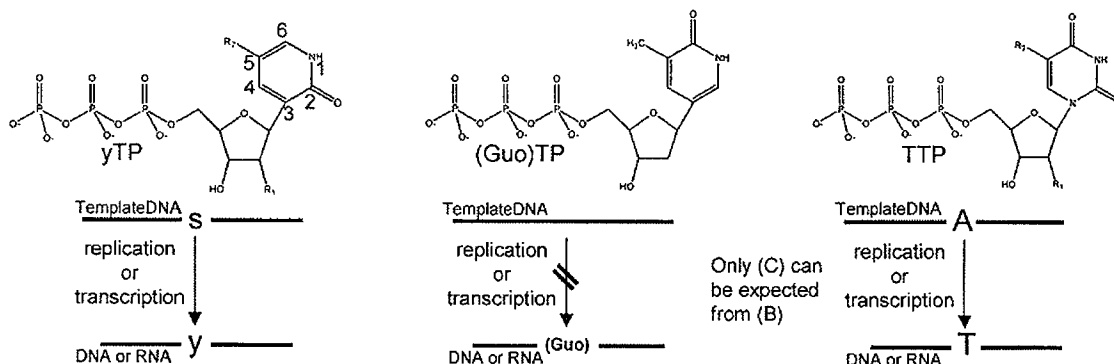
Ohtsuki *et al.* describe a method to synthesize the nucleoside derivatives of 5-methyl-2-pyridone. The method of Ohtsuki *et al.* comprises a step of binding 5-methyl-2-fluoropyridine with the ribose material. This is significantly distinct from the present method wherein the 5-position of 5-iodo-2-pyridine in the nucleotide derivatives is converted to a substituent selected from the group consisting of 1) to 4) described in Claim 2 of the present application. Ohtsuki *et al.* could be applicable only for 2-pyridone with only simple substituents, such as an alkyl group or alkynyl group at the 5-position. Therefore, the nucleoside derivatives of the present invention with complicated substituents can not be synthesized by the methods described in Ohtsuki *et al.*

Unexpected results of the present invention

A) The present invention

B) Guo et al.

C) Natural base: Thymidine (T)



s: 2-amino-6-thienylpurine

y: pyridine-2-one

x: 2-amino-6-(dimethylamino)purine

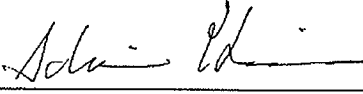
The nucleoside derivatives of the present invention can be incorporated into a specific position of nucleic acids by replication or transcription using a template DNA comprising an artificial base, such as "s" or "x", which specifically pairs with the 5-substituted pyridone (pyridine-2-one, y) in the polymerase reactions. The present invention is based on the following three technical ideas: (I) DNA or RNA polymerase can recognize and interact with the oxygen atom at 2-position in the pyridone; (II) The nucleoside derivatives of the present invention can also form base pairs with an artificial base, such as "s" or "x" because the 6-position in the pyridone of the nucleoside derivatives has a small hydrogen atom to accommodate the complementary large artificial s or x base; (III) The nucleoside derivatives of the present invention wherein 2-pyridone thereof has various complicated substituents at the 5-position.

Contrary to the subject invention, Guo *et al.*, "Inhibition of DNA Polymerase Reactions by Pyrimidine Nucleotide Analogues Lacking the 2-Keto Group," Nucleic Acids Research, 1998, Vol.26, No.8. p.1863-1869 (hereinafter "Guo *et al.*"), (B) discloses an idea corresponding to (I), only, and does not provide any suggestion to achieve the present invention. In the first place, Guo *et al.* describes experiments using nucleoside derivatives wherein the keto group at 2-position is deleted from thymidine (T). Therefore, Guo *et al.* only provides, at most, suggestion regarding relationship of the embodiment (B) to the embodiment (C) for the natural base pairs between A and T.

In addition, the present invention has identified that nucleoside derivatives having the 2-pyridone derivatives with various substituents at 5-position can be introduced into a specific position in DNA or RNA by replication or transcription mediated by artificial, extra base pairs. This is not possible with the teachings of either of Ohtsuki *et al.* or Froehler *et al.* The technical idea of (III) discussed above has first enabled application to replication or transcription using the specific artificial base pairs.

STATEMENT UNDER 18 U.S.C. § 1001

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By: 
Ichiro Hirao

Date: March 9, 2009